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INHIBITORY EFFECT OF INTERFERON ON RIBONUCLEIC ACID OF POLIOMYELITIS AND LOUPING ILL VIRUSES

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It has been established that the mechanism of the protective action of interferon is connected with its inhibiting influence on the reproduction of virus within the cell. However, at which stage of virus reproduction the activity of interferon is directed (replication of nucleic acids, synthesis of protein, composition of nucleic acids and protein) has not been revealed. Most probable is the influence of interferon on the process of replication of nucleic acids, though many authors refute the possibility of the direct influence of interferon on viral RNA (Ho Mont [6], Grossberg [4], Holland [5], Sommer, Prinzie, Denys [2], Shonne, Mayer, Cokol, Vilcek [8], and others [3, 9]).

In the present work evidence is presented of the direct influence of interferon on RNA, isolated from the poliomyelitis and louping ill viruses.

Materials and Methods

We used the louping ill virus (obtained from the Rockefeller Foundation in the USA in 1944) in the form of a 10% brain suspension of infected mice and the poliomyelitis virus type I, strain Magoni, enriched on primary tissue culture of human fetus kidney or on transplanted Detroit-6 culture.

After the interaction of the studied viruses, taken in a concentration of 1--3 lg ID_{50}, with interferon their residual activity was determined in diminishing 10-fold dilutions by means of intracerebral titration on mice (louping ill virus) or on a tissue culture of Detroit-6 (poliomyelitis virus). With each dilution of the poliomyelitis virus 4--5 test tubes, or 1 flask, were infected with a tissue culture, and with the louping ill virus - 3 mice each. The LD_{50} and TCD_{50} were calculated by the method of Reed and Mench (1938). For infection of the tissue cultures the virus was incubated with the cells for one hour, after which the nonadsorbed virus was removed by a double washing with a phosphate-buffer physiological solution (pH 7.2).

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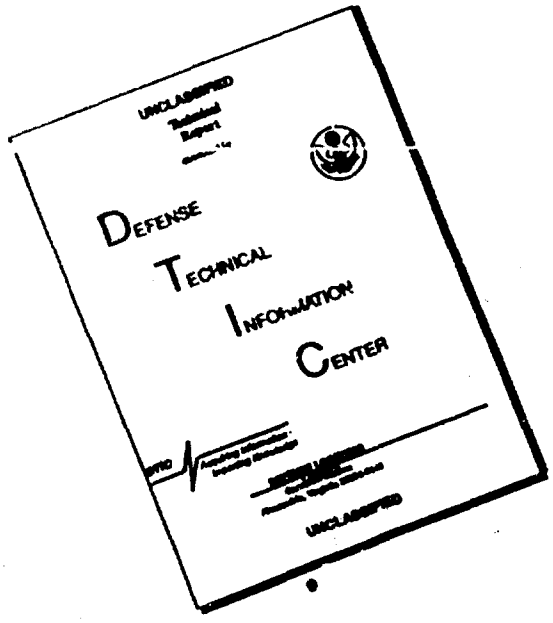

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RNA was isolated from the viruses by the method of Girer and Shramm (1956). The isolated aqueous preparation was alkalized with a 5% solution of soda to a pH of 7.0--7.2 and brought to the required ionic concentration with a 3-5 M solution of sodium chloride. For infection of tissue cultures we used RNA in a 1 M solution of NaCl, for the infection of mice - an 0.15 M solution of NaCl. Contact of RNA with the cells lasted for 20 minutes (Ellem, Kotler), after which the solution of RNA was removed by washing with a phosphate-buffer physiological solution. The tissue culture was infected with 1--2.5 lg ID_{50} of RNA, which approximated a working dose of the initial virus. Titration of the infectious activity of the resulting preparations of RNA was conducted in a similar manner to the titration of the corresponding viruses.

The specificity of RNA preparations was determined in control tests after a 30-minute contact with 10--30 μg/ml of RNase, which ensured the complete inactivation of RNA. We used a primary monolayer tissue culture of chick embryo and a transplanted culture of Detroit-6.

All the investigations were conducted with homogeneous interferon, obtained on a monolayer culture of chick fibroblasts, infected with the virus of Japanese encephalitis (strain No 47), in medium No 199. For cultivation of the virus we used a 1200 ml flask, into which 5,000 LD_{50} of virus was introduced. Four days after infection the cultural fluids from individual flasks were mixed, then cleared by 10-minute centrifuging at 3,000 rpm, after which the virus was inactivated by 1-hour heating at 60°. Activity of interferon was determined in a series of dilutions with a coefficient 2 on test tube cultures of chick fibroblasts relative to 50 TCD_{50} of the vesicular stomatitis virus. The average titer of interferon corresponded to a dilution of 1:32 and did not change after 6--12 months of storage at 4°. Interferon was used in a dose of 16 active units, i.e., in a dilution of 1:2.

In all the tests the control was cultural fluid with non-infected tissue culture of chick fibroblasts which had been subjected to similar treatment.

The following variants of interaction of viruses and their RNA with interferon were studied.

1. Interferon was introduced into the tissue culture 24 hours prior to its infection with viruses or their RNA. Prior to infection the liquid phase of the tissue culture together with the interferon contained in it was removed and the culture washed twice with a large volume of buffer solution.

2. Interferon was introduced into the tissue culture immediately after adsorption of virus or RNA.

3. Interferon was introduced 24 hours prior to infection, and then additionally immediately after adsorption of the infectious material. In the latter case the constant presence of interferon in the tissue culture was ensured.

4. The direct influence of interferon on infectious material was studied under the conditions of preliminary contact of interferon and RNA or homologous virus at 37° prior to the introduction of various dilutions of this mixture into the tissue culture. After a brief period of contact between them the mixture of viruses or RNA with interferon was titrated by the method described above. In the control the infectious material interacted with normal cultural fluid.

Results Obtained and Discussion

In series I of the tests we looked into the possibility of intracellular blocking of infectious RNA by interferon. For this purpose we studied the multiplication of virus in a tissue culture of chick fibroblasts, treated with interferon 24 hours prior to infection with louping ill virus (2 lg LD_{50}/0.03 ml) and its RNA (1.7 lg LD_{50}/0.03 ml). In the culture treated with interferon the virus was not revealed 24 hours after infection with the stated infectious material, while in the control tissue culture the quantity of virus comprised 1.5 lg LD_{50}/0.03 ml during infection with virus and 1.3 lg LD_{50}/0.03 ml after infection with RNA. In 3 days the content of virus in the test culture comprised 1.5 and 2.3 lg LD_{50}/0.03 ml against 4.5 lg LD_{50}/0.03 ml in the control (Table 1).

Thus, under the influence of interferon, introduced into a tissue culture of chick fibroblasts 24 hours prior to infection, there was a distinct slowing down of synthesis of virus both following the introduction of louping ill virus into the culture and also of its infectious RNA.

An inhibiting effect for interferon was observed also in respect to the infectious process induced by the RNA of the poliomyelitis virus on a culture of chick fibroblasts which were not sensitive to the poliomyelitis virus.

According to Holland [5], Yu. Z. Gendon [1], and others, RNA of the poliomyelitis virus, which had been introduced into a non-sensitive culture, causes only one cycle of multiplication of virus, since newly synthesized infectious viral particles are not able to take root in the new cells and cause infection.

Table 1

Dynamics of quantitative accumulation of infectious RNA and louping ill virus in a tissue culture of chick fibroblasts during interaction with interferon which was introduced 24 hours prior to infection

(a) Инфекционный материал	(b) Доза ($lg\ LD_{50/0.03\ мл}$)	(c) Обработана тканевая культура за 24 часа до заражения	(d) Концентрация вируса в $lg\ LD_{50/0.03\ мл}$, выявленная внутримозговым заражением мышей через		(g) Число $lg\ LD_{50}$ вируса, ингибированное интерфероном через 3 суток после заражения
			(e) 1 сутки	(f) 3 суток	
РНК шотландского энцефалита (h)	1,7	(j) Нормальная культуральная жидкость	1,3	4,5	0
		(k) Интерферон	0	1,5	3,0
Вирус шотландского энцефалита (i)	2,0	(j) Нормальная культуральная жидкость	1,5	4,5	0
		(k) Интерферон	0	2,3	2,2

Key: (a) Infectious material; (b) Dose ($lg\ LD_{50/0.03\ ml}$); (c) Tissue culture treated 24 hours prior to infection; (d) Concentration of virus in $lg\ LD_{50/0.03\ ml}$, exposed by intracerebral infection of mice in; (e) 1 day; (f) 3 days; (g)Amount of $lg\ LD_{50}$ of virus, inhibited by interferon in 3 days after infection: (h) RNA of louping ill; (i) Virus of louping ill; (j) Normal cultural fluid; (k) Interferon.

Titration of the poliomyelitis virus, induced by the introduction of RNA in a tissue culture of chick fibroblasts, was carried out on a tissue culture of Detroit-6 which was sensitive to the virus. In contrast to intact virus, infectious RNA induced the synthesis of poliomyelitis virus in a culture of chick fibroblasts. Regardless of the dose of RNA, used for the infection of cells of chick fibroblasts, the infectious activity was completely suppressed by interferon, if its introduction preceded the infection. If the interferon was introduced immediately after infection of the culture, suppression of RNA activity was not observed (Table 2).

For clearing up the problem of whether or not interferon was capable of the destruction or blocking of infectious viral RNA, tests were set up on the direct interaction of interferon with RNA from the viruses of poliomyelitis and louping ill. These were investigated simultaneously with homologous viruses taken in the corresponding doses. Results of the interaction in vitro at 37° were determined by titration on a sensitive tissue culture of Detroit-6 (for the poliomyelitis virus) or by intracerebral infection of mice (for the virus of louping ill).

Table 2

Inhibiting action of interferon on RNA of the poliomyelitis virus (strain Magoni type I) in interferon-homologous tissue culture of chick fibroblasts

(a) Доза РНК в lg $ИД_{50}$	(b) Изучаемый показатель	(c) Количество вируса в lg $ИД_{50}$ при внесении в тканевую культуру				Контроль (необработанная тканевая культура) (l)	
		(d) нормальной культуральной жидкости	(e) интерферона			РНК+РНК-аза (j)	10–100 $ИД_{50}$ вируса (k)
			за 24 часа до заражения (f)	сразу после заражения (g)	за 24 часа до и после заражения (h)		
1,3	Количество вируса в $ИД_{50}$ [1]	2,5	0	2,5	0	0	0
	Наличие ингибиции (m)		Полная	Нет (p)	Полная		
1,5	Количество вируса в $ИД_{50}$	2,5	0	2,3	0	0	0
	Наличие ингибиции		Полная	Нет	Полная		
2,0	Количество вируса в $ИД_{50}$	3,0	0	3,2	0	0	0
3,0	То же	4,0	0	—	0	0	0
	Наличие ингибиции		Полная	Нет	Полная		
1,0	Количество вируса в $ИД_{50}$	2,5	—	2,3	0	0	0
	Наличие ингибиции		—	—	Полная		

1. Concentration of virus was determined in 24 hours after infection by means of titration of cultural fluid on Detroit-6 tissue culture which is sensitive to the poliomyelitis virus.

Key: (a) Dose of RNA in lg ID_{50}; (b) Index studied; (c) Amount of virus in lg ID_{50} following administration into tissue culture; (d) of normal cultural fluid; (e) interferon; (f) 24 hours; (g) immediately after infection; (h) 24 hours before and after infection; (i) Control (untreated tissue culture); (j) RNA plus RNase; (k) 10-100 ID_{50} of virus; (l) Amount of virus in ID_{50} [1]; (m) Presence of inhibition; (n) Same; (o) Complete; (p) None.

The results of the interaction of interferon with RNA of the poliomyelitis virus are presented in Table 3. The control virus did not lose infectious activity after preliminary contact with interferon, while RNA completely lost its cytopathic activity after 5 minutes and more of contact with interferon at 37°.

Complete disappearance of infectious properties was also observed after the direct interaction of interferon with RNA of the louping ill virus along with preservation of activity of homologous virus under the same conditions (Table 4).

Table 3

Influence of preliminary contact at 37° of interferon with poliomyelitis virus and its RNA on their cytopathic activity in a tissue culture of Detroit-6

№ опыта	Инфекционный материал	Инфицирующая доза (lg LD_{50}/0,03 мл)	Исследуемые препараты	Время контакта при 37° (в минутах)	Титр вируса после контакта с указанными препаратами (lg ТЦД 50/0,3 мл)	Количество ингибированных инфекционных доз вируса (lg ТЦД 50/0,3 мл)
1	Вирус	1,7	Интерферон		1,7	0
			Нормальная культуральная жидкость	30	1,7	0
	РНК	1,0	Интерферон		0	1,0
			Нормальная культуральная жидкость	30	1,0	0
			РНК-аза		0	1,0
2	Вирус	1,5	Интерферон		1,5	0
			Нормальная культуральная жидкость	30	1,5	0
	РНК	1,0	Интерферон		0	1,0
		1,0	Нормальная культуральная жидкость	30	1,0	0
			РНК-аза		0	1,0
3	Вирус	1,0	Интерферон	5	1,0	0
			Нормальная культуральная жидкость		1,0	0
			Интерферон		0	2,0
			Нормальная культуральная жидкость	5	2,0	0
	РНК	2,0	РНК-аза	30	0	2,0
4	Вирус	1,5	Интерферон		1,5	0
			Нормальная культуральная жидкость	5	1,5	0
	РНК		Интерферон		0	1,5
		1,5	Нормальная культуральная жидкость	5	1,5	0
			РНК-аза	30	0	1,5
5	Вирус	1,5	Интерферон		1,5	0
			Нормальная культуральная жидкость	5	1,5	0
	РНК	2,0	Интерферон		0	2,0
			Нормальная культуральная жидкость	5	2,0	0
			РНК-аза	30	0	2,0

Key: (a) No. of test; (b) Infectious material; (c) Infecting dose (lg LD_{50}/0.03 ml); (d) Investigated preparations; (e) Time of contact at 37° (in minutes); (f) Titer of virus after contact with the stated strain (lg TCD_{50}/0.3 ml); (g) Amount of inhibited infectious doses of virus (lg LD_{50}/0.3 ml); (h) Virus; (i) RNA; (j) Interferon; (k) Normal cultural fluid; (l) RNase.

Table 4

Influence of preliminary 30-minute contact at 37° of interferon with louping ill virus and its RNA on their infectious activity, revealed by means of intracerebral infection of white mice

(a) № опыта	(b) Инфекционный материал	(c) Доза инфекционного материала (в lg LD 50/0,03 мл)	(d) Исследуемый препарат	(e) Результаты титрования на мышах после контакта в разведении			(f) Инфекционная активность после контакта с указанными препаратами (в lg LD 50/0,03 мл)
				−1	−2	−3	
1 (g)	Вирус	1,7	Интерферон (i)	3/3	1/3	0/3	1,7
			Нормальная культуральная жидкость (j)	3/3	1/3	0/3	1,7
(h)	РНК	2,3	Интерферон (i)	0/3	0/3	0/3	0
			Нормальная культуральная жидкость (j)	3/3	3/3	0/3	2,3
			РНК-аза (k)	0/3	0/3	0/3	0
2 (g)	Вирус	1,5	Интерферон (i)	3/3	0/3	0/3	1,5
			Нормальная культуральная жидкость (j)	3/3	0/3	0/3	1,5
(h)	РНК	1,5	Интерферон (i)	0/3	0/3	0/3	0
			Нормальная культуральная жидкость (j)	3/3	0/3	0/3	1,5
			РНК-аза (k)	0/3	0/3	0/3	0
3 (g)	Вирус	2,3	Интерферон (i)	3/3	3/3	0/3	2,3
			Нормальная культуральная жидкость (j)	3/3	3/3	0/3	2,3
(h)	РНК	1,7	Интерферон (i)	0/3	0/3	0/3	0
			Нормальная культуральная жидкость (j)	3/3	1/3	0/3	1,7
			РНК-аза (k)	0/3	0/3	0/3	0
4 (g)	Вирус	1,5	Интерферон (i)	0/3	0/3	0/3	1,5
			Нормальная культуральная жидкость (j)	3/3	0/3	0/3	1,5
(h)	РНК	2,0	Интерферон (i)	0/3	0/3	0/3	0
			Нормальная культуральная жидкость (j)	3/3	3/3	0/3	2,0
			РНК-аза (k)	0/3	0/3	0/3	0

Note: Numerator - number of sick mice, denominator - total number of mice in test.

Key: (a) No. of test; (b) Infectious material; (c) Dose of infectious material (in lg LD_{50}/0.03 ml); (d) Investigated preparation; (e) Results of titration on mice after contact in dilutions of; (f) Infectious activity after contact with stated preparations (in lg LD_{50}/0.03 ml); (g) Virus; (h) RNA; (i) Interferon; (j) Normal cultural fluid; (k) RNase.

In view of the inactivation of viral RNA following direct contact with interferon in the tests in vitro, the thought arose concerning the presence of admixtures of RNase in the cultural fluid of infected tissue cultures which was the substrate of the interferon utilized.

According to data from the literature, the amount of RNase in infected cultures does not change or is even reduced in comparison with the control of Kovacs [7] (1956).

Therefore the absence of activity in respect to viral RNA in the control tests with normal cultural fluid creates a foundation to connect our observations of suppression of infectious RNA in vitro with the participation of interferon.

Conclusions

1. Interferon, obtained by means of infection of cells of chick fibroblasts with the virus of Japanese encephalitis, displayed an inhibiting activity in respect to infectious RNA from the viruses of poliomyelitis and louping ill following direct action in vitro. Inactivation of RNA of the louping ill virus was established in tests on the intracerebral infection of mice, and the virus of poliomyelitis - on a culture of chick fibroblasts which were not sensitive to poliomyelitis virus.

2. The capacity of interferon to inactivate infectious viral RNA during direct interaction with it outside of a tissue culture makes it possible to assume that the same mechanism lies at the basis of the protective effect of interferon.

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